A STUDY OF THE SPORANGIA AND GAMETOPHYTES OF SELAGINELLA APUS AND SELAGINELLA RUPESTRIS.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XXXI.

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(WITH PLATES V–IX)

[*Concluded from p. 141.*]

GENERAL DISCUSSION.

CONSIDERING the phylogenetic importance of the group, the literature on the Selaginellaceae is surprisingly scanty. More especially is this true of that which treats of the development of spore and gametophyte. Some marked discrepancies in essential features have appeared in these papers, however, and show how much detailed life histories of more species are needed before further inferences are drawn as regards the relation of this group to others. Only one comparatively full account of a single species has yet appeared, Bruchmann's monograph on *S. spinulosa*, published in 1897, although he gives no account whatever of the development of the male gametophyte nor of the sporangia. He had much difficulty in obtaining normal spores,[1] and therefore was unable to follow the earliest stages of the prothallium in detail. In general, however, he agrees with Heinsen and Arnoldi that there is free cell formation within the spore, followed by periclinal and anticlinal cell walls, thus making a disk-shaped mass of cells in the apical portion of the

[1] "Uber die Keimung der Sporen habe ich zu zwei verschiedenen Malen Versuche angestellt. Das erste Mal sate ich sie gleich nach ihrer Reife (im August) auf Torf aus, hielt sie bestandig feucht und schutzte sie im Winter gegen Frost. Die erste Keimung weniger Sporen dieser Aussaat bemerkte ich erst nach zwei Jahren, weitere keimten dann in dritten Jahre, aber der grosste Teil zeigte selbst nach solcher Zeit keine Keimung."

spore. The upper layers of cells are much smaller and irregularly polyhedral in shape. No diaphragm is formed. Three tufts of trichomes grow from the upper surface of the prothallium, rend the exospore into three flaps along the ridges at the apex, and push them up out of the way of the developing archegonia. Only a limited number of the latter develop, some five to ten as compared with Pfeffer's report of thirty in *S. Martensii*. There are four rows of neck cells, with three cells in each row. Pfeffer reports but two cells in each of the four rows in *S. Martensii;* Campbell reports two in *S. Kraussiana*. Heinsen gives a list of eleven species: *S. Martensii*, *S. lepidophylla*, *S. Willdenoviana*, *S. denticulata*, *S. apus*, *S. erythropus*, *S. Helvetica*, *S. serpens*, *S. Douglasii*, *S. glauca*, *S. pilifera*, and says that he agrees with Pfeffer on this point. Bruchmann finds that although several embryos may start to grow, but one comes to maturity. The first division of the oospore of *S. spinulosa* is transverse to the axis of the archegonium, and the cell nearer the neck becomes a suspensor.

The most notable fact, however, is that this embryo has no foot. "Ein Fuss in dem Sinne, sie ihn z. B. *S. Martensii*, *S. Kraussiana* und andere Arten besitzen, fehlt." Whereas, the embryo of *S. apus*, as I have found, has no suspensor. After fertilization the cover cells of the archegonium close together, the walls of the neck cells thicken and turn brown. On the other hand, Pfeffer maintains that the neck of the archegonium of *S. Martensii* gapes widely during the entire embryonic development; and he represents the suspensor pushing up into the neck canal like a wedge. This latter method is the rule in most pteridophytes, and hence *S. spinulosa* together with *S. apus* and *S. rupestris* are exceptions.

In the last twenty years, as far as I know, but four other contributions have been made to a knowledge of these phases of the history of the Selaginellas. In 1871, Pfeffer published his paper "Die Entwickelung des Keimes der Gattung Selaginella." The extreme difficulty of interpreting the spore and prothallial structures, even with the aid of all the modern technique, makes

the description given by this author seem an extraordinary performance, and none the less that apparent discrepancies with other species may disappear upon reexamination. His account of *S. Martensii* differs from the foregoing in the following particulars: The megaspore when about one fourth its final size possesses two coats, exine and intine. Against the inner surface of the latter lies a thick layer of protoplasm inclosing a transparent fluid in which floats a large "nucleus." Near the apex "the protoplasm has a different appearance," but he was unable to interpret it. Obviously, he misinterpreted the so-called nucleus, which is the protoplasmic vesicle, and in all probability the peculiar appearance near the apex was due to the presence of the real nucleus. A dome-shaped diaphragm separates the gametophyte into two regions. This he thinks arises at the first division of the spore into two cells. The one toward the apex of the spore becomes subdivided into a tissue three layers thick in the middle, one layer at the periphery. The continuous division of the lower cell fills the basal portion of the spore with larger cells. The hollow spherical portion of the gametophyte below the disk he describes as becoming filled with "freigebildete Primordialzellen." He could not demonstrate the presence of nuclei in these cells, but regards it as probable. According to his statement, the growth of the prothallium occurs at two periods. The disk-shaped mass of cells appears before the spores are shed, the tissue below the diaphragm, the "secondary endosperm," afterward. Archegonia do not develop until the spores fall.

In 1894 Heinsen reexamined the species studied by Pfeffer. He fell into the same error as regards the interpretation of the protoplasmic vesicle and nucleus in the young megaspore. He was unable to determine the origin of the megaspores, other than that a tetrad arose endogenously in the spore mother cell. He denied the existence of a primary and secondary endosperm, and of a diaphragm, and corrected Pfeffer's misinterpretation of the food balls in the gametophyte cavity "as freely formed cells." Infrequently he found the archegonia formed in unshed spores.

The diaphragm which Pfeffer thought was the wall of the first division of the spore Heinsen regards as the plane of separation between the small cells at the apex of the gametophyte and the larger ones below, and not a wall. He also refutes Pfeffer's statement that ultimately the cell divisions completely occupy the basal region of the spore. Heinsen lays great stress on the supposed fact that the nuclei of the Selaginellaceae increase solely by direct division. He investigated material killed at all hours of the day and night, not only spores but vegetative tips, with special reference to establishing this point. He found a total absence of karyokinetic figures. As this statement, which if true would be most surprising, has not been refuted by later writers, it may be of interest to note a possible explanation of the error. In describing his methods of imbedding, Heinsen says that he killed his material by immersing some specimens for ten minutes in Flemming's fluid, others three minutes in a 1 per cent. corrosive sublimate solution, still others "mit gleich gutem Erfolge wante ich ein zweimaliges schnelles Eintauchen in kochendes Wasser an." It is doubtful whether any of the killing fluids thus employed had opportunity to penetrate the sporangium wall in so brief a contact; in which case his material died a lingering death through the washing and dehydrating processes. Under such conditions naturally there would be no traces of karyokinesis. The statement that he had as good results from two quick dips in boiling water as from the Flemming fluid is otherwise inexplicable. It is somewhat difficult to determine whether Heinsen means his statements to be general with regard to all the species he enumerates or not. His summary leads one to assume that the sequence of events there set down is true of the eleven species above named. There are certainly inaccuracies in many details if applied to *S. apus*.

Arnoldi in the *Botanische Zeitung* of 1896 followed in a brief article on "Die Entwickelung des weiblichen Vorkeimes bei den heterosporen Lycopodiaceen." He investigated *S. cuspidata elongata*, and gives a résumé of Heinsen's paper. He agrees with Heinsen on all points, thus reiterating the misinterpretation of

the spore content. Campbell in his *Mosses and Ferns*, published in 1895, gives an account of his investigation of *S. Kraussiana*. He too describes "the single large globular nucleus" (*i.e.*, the protoplasmic vesicle) of the megaspore. The diaphragm which exists in this species he explains as arising, not as Pfeffer thought from the first division of the spore, but from the thickening of the walls of the lowermost layer of cells of the disk at the spore apex. He finds "numerous small nuclei" scattered through the protoplasm of the spore cavity, and the protoplasmic layer thickens until it "completely fills the cavity of the spore." I have examined *S. Kraussiana* with reference to these points. The diaphragm is very evident and obviously formed in the manner described by Campbell. I can demonstrate no nuclei at any period in the spore cavity, nor any protoplasm. The protoplasm forms a layer next the wall, as in *S. apus*, and the vacuole within is full of food matter, at first a fluid, then an emulsion, and finally filled with granules and small balls of matter which stain like nuclei. The protoplasmic layer grows thicker, but never fully occupies the interior space as Campbell describes.

The latest contribution is that of Fitting (1900), "Bau und Entwickelungsgeschichte der Makrosporen von Isoëtes und Selaginella und ihre Bedeutung fur die Kenntnis des Wachstums pflanzlicher Zellmembranen." He corrects the error made by each of the above named writers in interpreting the parts of the megaspore. An attempt to discover the exact origin of the megaspores failed, due to the fact that the spore mother cell stained so deeply in all his preparations that the details of the evolution of the four spores could not be made out. He was unable to avoid shrinkage of cells. There follows a very detailed account of the development of the spore coats, in which he differs from Heinsen as regards their origin. He drew conclusions largely from living material examined in a salt solution. As I have said before, the interpretation of the origin of the spore coats seems to me largely a matter of theory, and one who has examined the young spores of *S. apus*, while inclosed in the sporangium and surrounded by sterile mother cells, is

skeptical of the results obtained by this method of investigation. He fails to comment on Heinsen's statement with regard to the method of cell division. Both Arnoldi's and Fitting's articles have been comparative studies of certain phases of the Selaginella life history with the same stages of Isoëtes. Thus, until the year 1900 we have not had a correct interpretation even of the parts of the megaspore.

MALE GAMETOPHYTE.—We are indebted to Millardet's memoir for our earliest knowledge of the male gametophyte. This work appeared in 1869, and almost no detail of importance has been added by later workers. In 1885 Belajeff repeated Millardet's work on the same species and corroborated all essential details. There has been no other during the last sixteen years until the present account given in this paper of *S. apus*. How much the discrepancies between the two are due to differences in methods of technique, rather than to specific characteristics, remains to be demonstrated. Millardet and Belajeff both examined the microspores of several species of Selaginella (*S. Kraussiana*, *stolonifera*, and *cuspidata*) in living condition, then added various reagents to the microscope slide, focusing through the spore coats or crushing them by pressure of the cover glass, to determine the phenomena taking place within. The material for the account of the male gametophyte of *S. apus* was killed, imbedded, sectioned, and stained without removing the spores from the strobilus. It will be seen that the main difference lies in the fact that in the earlier accounts there are eight cells described which constitute an antheridial wall; that later these cells disappear, and the sperm cell complex floats free in the cavity thus formed. Both authors state that they were unable to secure a cellulose reaction for the cell walls. On the other hand, I could demonstrate the presence of no such walls in *S. apus*. The first division of the spore results in two free cells. The first, according to Pfeffer's and Millardet's view, is the reduced vegetative part of the prothallium. The latter, which I have called the generative cell, divides at once into a complex of sperm cells. Is it possible that the protoplasmic films surrounding the vacuoles

containing food bodies were mistaken by these authors for cell walls? It is not quite certain that the species of Selaginella called *Kraussiana* in America is the identical one known by that name in Europe. The writer investigated the American form and found the sequence of events identical with those that obtain in *S. apus*. In attempting to repeat the methods employed by Millardet and Belajeff, it was impossible to distinguish through the spore wall the nuclei from the food granules, as both stained alike. Removing the exospore by shoving the cover glass to and fro was a tedious performance, whose results hardly repaid the effort. Moreover, it was impossible to manipulate the stains with enough precision to differentiate the nucleoli. As regards the spermatozoids, explicit statements are few. Belajeff figures those of *S. cuspidata* as biciliate bodies, somewhat elliptical in shape, pointed at the ends, with a slight spiral twist. Millardet gives substantially the same description, but no figures. In connection with his account he makes this reference to the work of M. Roze, who had published his observations in 1864:

Ainsi qu'on le voit, mes observations sur la forme des anthérozoïdes du *Selaginella Kraussiana* sont loin de s'accorder avec celles de M. Roze sur les anthérozoïdes des *S. Martensii* et *S. Galeottii*. Toutefois, comme il a en affaire à des espèces differentes de celle que j'ai étudiée, je m'abstiens de toute discussion sur ce sujet.

That there existed little information on the subject at this time (1869) is evidenced by the following statement:

Les seules observations que je connaisse sur la germination des microspores du genre Selaginella sont celles de M. Hofmeister et de M. Roze. Ces deux auteurs se sont bornés à mentionner le fait de la production, dans la spore, de cellules contenant chacune un anthérozoïde. C'est M. Hofmeister qui a constaté le premier l'existence, et la de ces animalcules; M. Roze a montré qu'ils sont biciliés.

So far as I can determine, the authors who have described spermatozoids of named species of Selaginella that they themselves have seen are as follows:

1862. Hofmeister.

1864. Roze first demonstrated two cilia in *S. Martensii* and *S. Galeottii*.

1869. Millardet thus describes the spermatozoids of *S. Kraussiana:*

Il m'a semblé qu'elle se colore en violet par le chlorure de zinc iodé; je n'ose toutefois l'affirmer. On voit en dedans d'elle un filament roulé en hélice, soit à droite, soit à gauche, de façon à faire environ deux tours complets; l'une de ses extrémités est occupée par le granule réfringent d'amidon que je viens de signaler et par suite extrêmement visible; l'autre est à peine distincte. La première constitue la partie postérieure du corps de l'anthérozoïde, elle décrit un cercle plus étroit que le reste de l'animalcule; on voit d'habitude à côté d'elle quelques granulations à peine appréciables. Les cils semblent placés l'un à côté de l'autre; on les distingue difficilement du corps. . . . . Ces détails serviront à l'intelligence des différentes formes des spermatozoïdes. Dans leur état de développement complet, ils sont entièrement débarrassés de leur vésicule et de la membrane de leur cellule mère, et présentent à l'extrémité postérieure un corpuscule d'amidon; il est resté dans la vésicule où on le retrouve; c'est là une seconde forme. Une troisième est constituée par les animalcules qui portent avec eux une vésicule; une quatrième, par ceux qui ne sont qu'incomplètement dégagés de leur cellule mère.

Dans la forme la plus dévéloppée, le corps est presque droit et décrit une spirale à peine sensible. Sa longueur dans cet état est 0.018 ^{mm} environ. Il s'amincit graduellement depuis l'extrémité postérieure jusqu'à la naissance des cils: son épaisseur maximum ne dépassé pas 0.0007 ^{mm} environ. En avant il se bifurque et se termine ainsi par deux cils très-tenus, deux fois aussi longs le corps.

1871. Pfeffer described the male gametes of *S. Martensii*, and *S. caulescens*.

1885. Belajeff examined *S. cuspidata*, *S. laetevirens*, *S. Martensii*, *S. caulescens*, *S. stolonifera*, *S. Kraussiana*, and *S. Poulteri*. Of these he gives figures only of the spermatozoids of *S. stolonifera*.

Thus, in the thirty-nine years since the speramatozoids of the Selaginellaceae were first described, there have been only four contributions upon the subject in which the spermatozoids of but eight species are described from direct observation vouched for by the writer.[3] The difficulty of determining

[3] 1. *S. Martensii:* Roze, 1864; Pfeffer, 1871; Belajeff, 1885.
2. *S. Galeottii:* Roze, 1864.
3. *S. Kraussiana;* Millardet, 1869; Belajeff, 1885.
4. *S. caulescens:* Pfeffer, 1871; Belajeff, 1885.
5. *S. cuspidata:* Belajeff, 1885.
6. *S. laetevirens:* Belajeff, 1885.
7. *S. stolonifera:* Belajeff, 1885.
8. *S. Poulteri:* Belajeff, 1885.

the structure of bodies so extremely small can hardly be overestimated.

With either a Bausch & Lomb $\frac{1}{12}$ oil immersion objective and $\frac{3}{4}$ ocular, or a Zeiss oil immersion 2^{mm} and ocular 3, I was able to make out the spermatozoids only by their characteristic rotary movements as they left the microspore of *S. rupestris*. Their spiral form and attached vesicles were facts determined rather by interpreting appearances by those I had definitely seen in apparently similar but larger bodies, than by actual observations. The spermatozoids of *S. apus* are somewhat larger, and I feel that there is less likelihood of error in describing them. It is obvious that a more critical examination of many species is needed before much weight be placed upon the so-called aberrant forms of the Selaginella spermatozoids in tracing the phylogeny of the group.

THE DEVELOPMENT OF THE SPORANGIA.—The two most recent and important papers on the development of the sporangium are Goebel's (1880) and Bower's (1894). I shall quote verbatim Bower's summary of results from Selaginella:

1. The sporangium is eusporangiate, and arises from the tissue of the axis, above the subtending leaf; the position varies in different species.

2. The origin of the sporangium is similar to that of Lycopodium, and especially resembles *L. inundatum*, to which species the mature sporangium also is similar in form.

3. Two primary archesporial cells are usually present in each radial section, and these are derived, as in *L. inundatum*, from segmentation of two distinct cell-rows; as seen in tangential section, the archesporium is referable to three or four such cell-rows.

4. The first periclinal divisions in these cell-rows do not always define the archesporium finally; subsequent periclinal divisions may result in addition to the central mass, as has been proved for Equisetum; but here the addition is less regular.

5. The tapetum results from tangential division of the outermost cells of the central mass; the greater part of it originates as described by Goebel.

6. The tapetum is thus a sterilized part of the potential sporogenous tissue; a further example of sterilization is seen in the megasporangium, where all the sporogenous cells are disorganized, excepting the one mother cell of the megaspores.

7. Abortive sporangia are to be found at the base of the strobilus as in many species of Lycopodium.

With regard to the derivation of the sporogenous tissue, repeatedly radial sections show a distinct plane of segmentation separating the sporogenous tissue into two such definite regions that it is difficult to avoid the conviction that each complex is the progeny of two independent cells. The term "archesporium" is used by Bower to signify the lower cell or cells. I have used the term to indicate the superficial cell itself. With regard to the fourth statement, my observations on *S. apus* do not agree. With the fifth and sixth statements, as regards the origin of the tapetum, my observations are in accord. The seventh statement I find true of *S. rupestris*, but not of *S. apus*.

In no particular do my preparations agree with Campbell's figures of the sporangial development of *S. Kraussiana*.

Of no little interest is the incomplete septum in the microsporangium of *S. rupestris*, which arises above the pedicel. In vertical section, the resemblance to the celebrated fossil *Lepidodendron Braunii*, as figured by Bower (see his *plate 48, fig. 100*), is very marked. He discusses in the accompanying text the possible function of this region of the sporangium. "Possibly," he says, "these extensions of sterile tissue may have facilitated the nutrition of the developing spores, or they may perchance have contributed to the mechanical support of the sporangial wall." In connection with the former theory, it is noteworthy that this septum is found only in the microsporangium, where some of the spores are remote from the tapetum. A feature that may have some bearing on this point is the fact that the megaspore mother cell, so far as I have observed, is always near the periphery of the sporogenous mass and never at the center, a favorable position to secure nutrition from the tapetum. Scott in his *Studies in fossil botany*, 1900, says, *à propos* of this feature in *Lepidostrobus Veltheimianus*, "this structure may be compared with the trabeculae of Isoëtes. It is best shown in the microsporangium, but may have originally been present in the megasporangia also."

Of even greater interest is the simultaneous discovery of a paleozoic lycopod each of whose sporangia contains a single megaspore or embryo sac, which presumably was fertilized while still attached to the plant, with the condition that I have described above that obtains in *S. rupestris* and *S. apus*. Of the integument that grows up about the sporangium of the *Cardiocarpon anomalum*, described by Scott, leaving at the top an open slit-like micropyle, there is no trace I think, except that in *S. rupestris*, quite late in the development, after the embryo has formed, the megasporangium becomes sunken in a shallow pit formed by the cushion-like upgrowth of the sporophyll around the pedicel. This outgrowth hedges in the ligule with the sporangium, and may be homologous with the Lepidostrobus integument. Scott's statement, therefore, that "the recent discovery of Lepidostrobi with integumented, seed-like sporangia, in which only one megaspore came to perfection, shows that some paleozoic members of the group went far beyond any of the living representatives in the differentiation of their reproductive organs," needs modification in view of the fact that *S. rupestris* normally at the present day produces seed-like sporangia with well developed embryos.

SUMMARY.

1. In both *S. apus* and *S. rupestris* the sporangium frequently, if not always, may be traced to a single superficial cell, the archesporium.

2. The sporogenous tissue may arise in two ways. First (*S. rupestris*), from the single hypodermal cell formed by the archesporium being divided by a periclinal wall, thus producing a wall and a sporogenous cell. Second, by the archesporium (which in this case is assumed to consist of two independent superficial cells) dividing into four cells by a periclinal wall, the two hypodermal cells thus formed developing the sporogenous complex. In each case the epidermal cells form the sporangium wall. It is possible but not demonstrated that the second case may be a phase of the first wherein the original superficial archesporial cell divided by an anticlinal wall.

3. The tapetum is formed in part from the sporogenous cells near the exterior of the mass, and in part from adjacent vegetative cells which come to form a more or less regular layer. It is defined very early, and is active and glandular up to the period that the spores have attained their full size, then becomes reduced to a thin epithelial-like layer lying against the sporangium wall.

4. Microsporangia and megasporangia are indistinguishable before the spore mother cells are differentiated. At this stage, in the case of the megasporangium, one or rarely two cells become more conspicuous and divide into spores. If microspores are to be formed, the majority of the cells continue dividing. After the megaspore mother cell is differentiated, all other cells in the megasporangium cease dividing.

5. In *S. apus* four megaspores arranged tetrahedrally are formed within the spore mother cell. In *S. rupestris* four spores may develop in the same manner, only one or two of which may come to maturity; or, most commonly, there may be but a single division of the mother cell nucleus, in which case there are but two spores formed; or again, there may be a redivision of one only of the two daughter nuclei, resulting in three spores of which only one or two attain maturity. Isolated cases have been met with where but one megaspore was formed. As there were no signs of other aborted spores, presumably the megaspore mother cell never divided, but became directly the single megaspore. In no case have more than two spores in a single megasporangium been found which were fertile, and very frequently only one.

6. The megaspore of each species has three distinct coats, the exospore, mesospore, and endospore. The former originates on the inner face of the spore mother cell membrane, and when first distinguishable is a film of unequal thickness. This either directly or indirectly gives rise to the exospore. A thick layer develops between the exospore and the protoplasmic vesicle, which later in its history separates into two layers, the mesospore and the endospore.

7. The female gametophyte is formed by free cell division of the megaspore, the nuclei dividing by indirect division. These nuclei are confined to the apical portion of the spore. Several layers of nuclei are formed by repeated tangential and radial divisions so that there are six or seven in the apical region and one in the basal. Areas of cells are blocked off by protoplasmic radiations passing from the apex outward and inward. The walls of the cells are produced by nuclear plates in the final division. There is no diaphragm, and at no stage of its development are there nuclei in the lower or the central portions of the gametophyte, which at first contains liquid, and finally a semi-solid mass of granular matter.

8. A cushion of cells protrudes through the tripartite cleft in the exospore at the apex. From cells in the upper row of this cushion a limited number of archegonia develop. The cells of this region are markedly smaller than those in the other regions of the gametophyte. No part of the archegonium protrudes from the general level except the cover cells.

9. The megaspores and gametophytes are nourished by matter secreted by the tapetum and passed through the spore mother cell membrane, which persists until the spores are nearly half grown.

10. The microspores develop in a fashion analogous to the megaspore. A large percentage of the mother cells form tetrads, the largest proportion of which are aborted at this stage.

11. The male gametophyte of *S. apus* consists of a single prothallial cell and an ovoid naked mass of potential sperm cells arranged in two groups. There is no antheridium, nor is there a wall which separates the prothallial from the sperm cells.

12. The spermatozoid of *S. apus* is a spirally coiled body; that of *S. rupestris* is of similar shape but much smaller. The presence of cilia in either was not demonstrated.

13. The megasporangia and microsporangia of both species open by definite lines of dehiscence.

14. Fertilization in both species occurs while the spores are

unshed and the sporangia are still attached to the strobilus. At this period the strobilus ceases to form new sporangia. The strobilus of *S. rupestris* retains its physiological connection with the plant until the embryo has produced cotyledons and a root.

15. In the early autumn *S. apus* sheds all strobili whether fertilization has occurred or not. *S. rupestris* retains its stroblii throughout the winter and fertilization occurs in the spring.

16. *S. apus* forms twelve to fifteen megasporangia in each strobilus, all of whose spores are generally fertile. The comparatively limited number of embryos formed is due probably either to the limited number of fertile microspores, or to mechanical difficulties in the way of fertilization, possibly both. *S. rupestris* produces strobili far in excess of the number of purely vegetative branches, the majority of which develop only sterile spores except under very favorable conditions. The sterile spores are shed profusely during the summer, and the strobili which remain on the plant throughout the winter retain their power of apical growth. The first sporangia formed in the early spring contain microspores. There is but a limited number of these; then follows under favorable conditions either fertilization or the development of megasporangia, which continues until checked by the development of embryos in the older regions of the strobilus, or in case fertilization fails by the approach of winter.

TECHNIQUE.

The tender tips of the strobili offer no special difficulties in preparation for the microtome, but the older sporangia, especially the closely compacted strobili of *S. rupestris*, are exceedingly resistent to the entrance of reagents, and the hardened epidermis of the sporophyll, combined with the thick walls of sporangia and spores, render sectioning difficult. Certain stages of the spores are peculiarly liable to collapse. These technical difficulties probably explain many gaps in our knowledge. The following method was followed in preparing the material for this investigation.

KILLING AGENTS. — Flemming's weaker fluid, Hermann's chromacetic acid, and bichromate of potassium combined with acetic acid, gave the best results. Corrosive sublimate, absolute alcohol, picric acid, Merkel's and Perenyi's fluid were tried, but the results were unsatisfactory in that I failed to get such successful staining after their use.

These fixing fluids were used boiling hot. In the case of the Flemming, the chromic acid and water were brought to the boiling point, the osmic and acetic acids quickly added and the mixture poured over the strobili which had previously been removed from the plants. The vessel was covered tightly and the contents allowed to cool gradually to a temperature of 30° C. The spores were fixed in twenty four hours, but often were left in the killing fluid two or three days with no deleterious effects. Water of approximately the same temperature (30° C.) was used for washing, in very large quantities, and changed frequently for two days. Sometimes it was more convenient to use cold running water, in which case three days' washing was necessary to remove all acid. The dehydrating process was equally gradual: twelve hours each in 10 per cent., 20 per cent., 30 per cent., 50 per cent., 75 per cent., and 80 per cent. alcohol respectively. In 95 and 100 per cent. three days each, and the alcohol changed each day. Xylol was added to the specimens with even more caution, as an examination of material from day to day during these manipulations disclosed the fact that at this point danger of collapse was greatest. Six intermediate grades between absolute alcohol and pure xylol were employed, each for twenty-four hours. The strobili remained in pure xylol until they became transparent, which frequently was not for a week, dependent upon the age of the spores. When this condition was obtained, the xylol was replaced by fresh, and small bits of soft paraffin (melting point 30° C.) were added gradually as long as they would dissolve at the temperature of the room. This usually required two days. Thence the vessel was removed to the bath (temperature 40° C.), which was raised slowly to 54° C., soft paraffin being added

during the time up to the point of saturation. At this time the cover was removed from the vessel to facilitate the evaporation of the xylol, and harder paraffin added in small quantities at intervals. At the end of two or three days the temperature was raised to 65° C., and maintained for one week, after which time the xylol had evaporated, and the strobili were infiltrated with paraffin.

At first, on the supposition that such protracted exposure to reagents and heat would injure the more delicate very young sporangia, the tips of strobili were removed, carried through the various media in much less time, and subjected to a temperature of 50° C. for about ten hours only. Comparisons later showed that these precautions were unnecessary, and that the longer exposures produced better results, even in the apical cells. Moreover, having a strobilus cut *in toto* was of the greatest value in interpretation. Further experiments prove that considerable latitude in the direction of longer periods of immersion in the various fluids are not injurious, but all attempts to hasten results by shorter exposure than the period stated above were unfortunate. By an oversight, at one time the temperature rose in the bath three successive nights to 75° C., but with no injurious effects. Some difficulty was experienced in imbedding. The paraffin was inclined to shrink away from the rough surface of the spore wall, which caused the sections to drop out of the paraffin ribbon when transferred to the slide. The difficulty was overcome by removing the strobili to the imbedding dish from the bath, letting them cool off slightly (to about 40° C.), pouring on paraffin at a temperature of 80° C., then cooling as rapidly as possible in ice water. No further trouble was experienced and the strobili could be sectioned without difficulty.

STAINS.—The best stains for the youngest stages of sporangia are iron haematoxylin (Haidenhain method), and the so-called Flemming triple stain—saffranin, gentian-violet, and orange G. Frequently gentian-violet and orange G were employed without saffranin; for the gametophyte development,

after the appearance of the proteid matter in quantities that obscured the other cell features, cyanin and erythrosin, after treating the sections on the slide with dilute sulfuric acid and chloroform, produced very satisfactory results. The power to take up the stains was retarded in the case of the proteid granules, whereas the nuclei and cell walls were more readily and more brilliantly dyed after this treatment. Karyokinetic figures were especially clear.

It remains my pleasant duty to express my thanks to Dr. John M. Coulter, and to other members of the Botanical Department of the University of Chicago, for many suggestions offered in the course of this investigation. To Mr. E. J. Canning, Head Gardener of the Botanic Gardens of Smith College, I am deeply indebted for aid in collecting and growing material.

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EXPLANATION OF PLATES V–IX.

All figures were drawn with the aid of a camera lucida and a Bausch and Lomb microscope, and were reduced to one fourth their original size in reproduction.

PLATE V. Selaginella apus. Oc. ¾, oil immersion $\frac{1}{12}$.

FIG. 1. Vertical median section of tip of strobilus showing the apical cell, a young sporophyll whose apical cell is just established, an archesporial cell (shaded), and a two-celled sporophyll (distinguished by black nucleoli).

FIG. 2. Radial section of portion of strobilus showing sporophyll and two superposed cells (shaded), resulting from a transverse division of the archesporial cell. The superficial cell by repeated division in one plane

eventually forms the initial sporangium wall. The hypodermal cell is the initial sporogenous cell. The first cell of the ligule is distinguished by a black nucleolus.

FIGS. 3 and 4. Radial section of portion of strobilus showing two superficial archesporial cells (shaded).

FIG. 5. Radial section of strobilus showing three-celled sporophyll (near apex), a two-celled sporangium (shaded) near the base of the subtending sporophyll, and the early stage of the vascular system.

FIG. 6. Radial section of strobilus showing four-celled sporangium possibly formed by two archesporial cells which divided by transverse walls. A stage subsequent to *figs. 3* and *4*.

FIG. 7. Radial section of older sporangium. Sporogenous cells are shaded, and are represented with black nucleoli.

FIG. 8. Vertical section of sporangium showing definite radial arrangement of cells and plane of cleavage dividing it into two groups of cells, each the progeny of one of the two superficial archesporial cells shown in *figs. 3* and *4*.

FIG. 9. Vertical median section of sporangium more advanced. Sporangium wall at this stage consists of one layer.

FIG. 10. Median transverse section of sporangium showing the sporangium cells, the tapetum, the inner and the outer layers of the sporangium wall.

FIG. 11. Section of sporangium after the spore mother cells are established. In this stage the sporogenous cells are distinguished by two conspicuous masses of granular matter which lie against the nucleus.

FIG. 12. Group of sporogenous cells in synapsis stage. Probably microspore mother cells.

FIG. 13. Detail of small portion of megasporangium showing megaspore mother cell (distinguished by the lumps of matter on either side of the nucleus) lying near the glandular tapetal cells. Two sterile sporogenous cells are at the left and below the megaspore mother cell.

FIGS. 14–24. Stages in the development of the megaspores. *Fig. 16* shows the characteristic sextuple spindle stage previous to the separation into spores, as seen in *fig. 18*. The four nuclei pass from the bases to the apices of the newly formed spores, as shown in *figs. 18–22*.

FIGS. 25–28. Details of cell division in the final stages of development of the female prothallium. *Fig. 25* represents a portion of a section of the stage represented in *fig. 49* to show thickness of protoplasmic vesicle and the invasion of fibrillae blocking off the separate nuclei.

FIG. 29. Section of archegonium. The egg and ventral canal cell are lying in a plane perpendicular to the long axis of the archegonium. The egg is the larger cell with black nucleus.

FIG. 30 Section of archegonium in which the egg lies below the ventral canal cell, and the neck canal cell above it. Two tiers of neck cells are drawn.

FIG. 31. Stage younger than that shown above, before neck cells are cut off.

FIG. 32. Archegonium with egg and ventral canal cell laterally placed. No trace of neck canal cell. Neck consists of four tiers of cells, each tier comprising an upper (superficial) cover cell, and one neck cell. The cells abutting on the venter belong to the prothallium.

FIGS. 33 and 34. Mature archegonia. In *fig. 33* a spermatozoid is lying on the receptive spot of the egg. *Fig. 34* shows a one-celled embryo.

PLATE VI. Selaginella apus. Oc. 2, oil immersion $\frac{1}{12}$.

FIG. 35. Section of young megasporangium at earliest stage when megaspore mother cell is distinguishable from sterile cells. For detail see *fig. 13*.

FIG. 36. Section of older stage of megasporangium. Megaspore mother cell has moved near center of sporangium.

FIG. 37. Oblique section of megasporangium showing young tetrad, and sterile mother cells, floating in slime composed in part of disintegrated sterile cells and in part of a secretion poured out by tapetum.

FIG. 38. Section of somewhat older stage of megasporangium containing group of four megaspores (only three are represented). Each spore consists of an outer coat, the exospore; a median layer which is later differentiated into the mesospore and the endospore; a vesicle of protoplasm containing a limpid fluid and possessing a very small nucleus. The spaces intervening between vesicle and median layer and between the latter and the exospore are filled with fluid.

FIGS. 39–41. Successive serial sections of a tetrad, to illustrate the fact that the exospore is a continuous envelope common to the group of spores and splits as they move apart.

FIG. 42. Section of group of megaspores but little older than those in *fig. 38*. Filamentous processes shown between spores. The mother cell membrane, beyond whose boundary the radiations do not pass, envelops the tetrad.

FIG. 43. Section of two megaspores more advanced. The spore at the left is cut nearly in half from apex to base, that at the right is a slice across the apex. Protoplasmic vesicle grown but little larger than in *fig. 42*. Spine-like processes upon exospore in connection with radiations. Mother cell membrane still evident.

FIG. 44. Section of the female gametophyte showing the protoplasmic vesicle with two nuclei. The radiations extend across the spaces between vesicle and median layer, between median layer and exospore, and between the latter and the original spore mother cell membrane.

FIG. 45. Section of female gametophyte whose vesicle has five nuclei and no cell walls. The exospore has grown to the size of the megasporangium as shown in *fig. 38*. The median layer has divided into the endospore (represented in section as a broad black band), and the mesospore which can be distinguished only as a delicate layer (represented by a single line) just without the endospore. The exospore is of spongy appearance and is still in contact with the persistent spore mother cell membrane. Fluid, presumably protoplasmic in nature, fills intervening spaces.

FIG. 46. A transverse median section of a female gametophyte in "film" stage. The megaspore, endospore and protoplasmic vesicle (studded in its apical region with large free nuclei) have stretched to the dimensions of the exospore, against whose inner surface they form a layer consisting of three delicate lamellae. The central vacuole is filled with clear fluid. The same stage is shown in *figs. 47* and *48* the former representing a surface view, the latter a median section.

PLATE VII. Selaginella apus.

All figures except 56 and 58 drawn with oc. 2, obj. $\frac{1}{6}$. *Fig. 56* is drawn with same combination as plate V; *fig. 58* with oc. 2, obj. $\frac{2}{3}$.

FIG. 47. Surface view of the protoplasmic vesicle as if seen through spore walls.

FIG. 48. Vertical median section of the same stage. The nuclei are massed in the apical region and the interior of the vesicle is occupied by a vacuole.

FIG. 49. Surface view of the gametophyte at the moment when protoplasmic fibrillae appear in the apical region and radiate over the surface of the gametophyte. The nuclei in the lower part of the figure are in process of division. The vesicle has increased in thickness (cf. *fig. 50*), and is beset with proteid granules. Contents of vacuole a homogeneous liquid.

FIG. 50. Median section of same.

FIG. 51. Median vertical section of female gametophyte. Protoplasmic vesicle much thicker in apical region where the nuclei are disposed in several layers diminishing to a single one in the equatorial region. Fibrillae permeating the nucleated portion have outlined indefinite areas but there are no cell walls. Contents of vacuole an emulsion.

FIG. 52. Section showing a portion of the megasporangium with female gametophyte *in situ* to show the differentiation of the wall. In the lowermost part of the figure the fragile cells of the area of dehiscence are seen in cross section. The inner layer of the wall probably supplies the outer with nourishment. The vestiges of the tapetum are upon its inner face, as a pavement layer, and a few sterile mother cells lie at the left next the wall.

FIG. 53. Vertical median section of female gametophyte showing the differentiation of cells into an apical superficial layer from which develop

archegonia, and a lower vegetative region. The delimitation of the archegonial region is shown by the trefoil shaped cleft seen in the lowermost spore of *fig. 58*.

FIGS. 54 and 55. Section of female gametophyte showing archegonia. Details of the process of division in the unshaded cells of *fig. 54* are represented in *figs. 26–28*.

FIG. 56. Two embryos, one of two cells, the other of three, lying side by side in one venter. All walls shown are parallel to the axis of the archegonium. Compare relative position of these embryos with that of the egg and ventral canal cell in *figs. 29, 32*.

FIG. 57. Embryo *in situ*. The root lies toward the apex of the gametophyte. At the right the club-shaped foot of large cells is embedded in the prothallial tissue. The embryo is bent with respect to its long axis so that the stem apex and cotyledons are brought nearer the root than appears in the drawing, which is a reconstruction from serial sections. The two leaves with their relatively large ligules are developed successively and envelop the tip of the stem (shown by dotted line). The embryo is still within the spore wall and sporangium.

FIG. 58. Vertical section of megasporangium containing three female gametophytes. The lowermost shows the trefoil-shaped cleft in the apical region of the spore wall, exposing the archegonia. The two other oblique sections are not so advanced in development. The pad of tissue at the base of the sporangium lies above a region of storage cells in contact with the vascular bundles of axis and sporophyte.

PLATE VIII. Selaginella apus.

All drawings made with oc. $\frac{3}{4}$, oil immersion, obj. $\frac{1}{12}$, excepting 60–62. Figs. 60, 61 were drawn with oc. 2, oil immersion, obj. $\frac{1}{12}$. Fig. 62 with oc. $\frac{3}{4}$, obj. $\frac{2}{3}$.

FIG. 59. Section of microsporangium, showing microspore mother cells, tapetum, inner and outer layers of sporangium wall.

FIG. 60. Median vertical section of microsporangium with mother cells in synapsis stage. Sub-archesporial pad developing at base of sporangium.

FIG. 61. Slightly oblique section of microsporangium showing microspores in groups of four (tetrads).

FIG. 62. Median vertical section of microsporangium to show sporangium wall at maturity. Dehiscence region cut across at apex. Sporangium opens in two valves, the subarchesporial pad at top of pedicel serving as a fulcrum.

FIGS. 63, 64. Microspore mother cells during first division of nucleus.

FIGS. 65, 66. Sextuple spindle stage with nuclear plates.

FIG. 67. Exterior view of young microspore.

FIG. 68. Section of tetrad, showing continuity of exospore around the four microspores, and spore mother cell membrane enveloping the tetrad. The protoplasm is a thin vesicle with a single nucleus, parietally placed, and surrounding a large central vacuole.

FIG. 69. Exterior view of mature microspore showing pebbled exospore.

FIG. 70. Vertical section of male gametophyte after first division of spore into generative and vegetative cells.

FIG. 71. Male gametophyte as seen by focusing through spore coats. The large generative nucleus lies near the wall, the vegetative cell not in focus.

FIG. 72. Male gametophyte showing generative cell in section.

FIG. 74. Male gametophyte showing lenticular vegetative cell at left. The remainder of the content constitutes the generative cell with a centrally placed nucleus. Masses of deeply staining proteid matter appearing.

FIG. 75. Section of male gametophyte in which masses of proteid matter have increased in number. Vegetative cell appressed to wall at left.

FIG. 76. The protoplasm is aggregated around the centrally placed generative nucleus and has sent out radiating processes, connecting it with a peripheral layer. The vacuoles thus isolated are filled with a semi-fluid granular matter.

FIGS. 77, 78. A common appearance, where a large symmetrical mass of proteid matter (solid black) in the central vacuole may be mistaken for a nucleus.

FIG. 79. First division of generative nucleus. Persistent vegetative cell appressed to wall in lower portion of figure.

FIGS. 80–87. Phases of the early divisions leading to the spermatozoid mother cells.

FIGS. 88–95. Sections in various planes showing the two cell complexes and their relation to the dissolving proteid masses by which they are enveloped. The masses of food matter are separated by strands of protoplasm in *figs. 88, 93, 95*.

FIG. 96. Exterior view of male gametophyte displaying trefoil shaped cleft in apical portion. The complexes of spermatozoid mother cells protrude through the gap.

FIG. 97. Male gametophyte with spermatozoid mother cells as seen by focusing through spore coats. The unusually persistent vegetative cell is represented lying above the mother cells.

FIGS. 98, 99. Male gametophyte at maturity. The endospore protrudes like a short pollen tube through a gap on the ruptured exospore, and contains a slimy homogeneous fluid which later is discharged with the spermatozoids.

FIG. 100. A transverse section of a female gametophyte displaying the

eggs and spermatozoids. The necks of the archegonia have been sliced off somewhat obliquely.

PLATE IX. Selaginella rupestris.

FIG. 101. Apex of a strobilus in radial section. A single archesporial cell (shaded) at the base of a sporophyll. The cell with black nucleus is the initial cell of the next younger sporophyll of the same rank.

FIG. 102. First division of archesporial cell into a hypodermal and a superficial cell.

FIG. 103. The superficial cell of the first division has divided into three wall cells, and the hypodermal cell into two sporogenous cells, forming a wedge-shaped mass.

FIG. 104. Radial section of portion of strobilus. The largest sporophyll has ceased to grow at the apex which is converted into a branched spine. The initial cells of the ligule at the base of the sporophyll are represented with black nuclei. The tissue is rupturing to form air chambers in the basal region. The sporangium has been outstripped in growth by the sporophyll next above it. The two shaded cells in the upper part of the figure represent the same stage of sporangium as seen in *fig. 102*.

FIG. 105. Section of sporangium. The wall is nearly completed and the tapetum is becoming differentiated from the sporogenous complex of cells. The limits of the latter in the basal region not so clearly defined as repre sented in the figure.

FIG. 106. Detail showing two megaspores and several sterile mother cells.

FIGS. 107–117. Phases undergone by megaspore mother cells preceding divisions into spores.

FIG. 118. First division of megaspore mother cells into two spores.

FIG. 119. Section through sister megaspores. A portion of the sculptured exospore is removed from the upper surface of each, showing the protoplasmic vesicle with a small nucleus floating in liquid. The spore mother cell membrane envelops the two spores.

FIG. 120. Section showing incomplete division of the protoplasm of two megaspores. The nucleus of the spore mother cell has not divided.

FIG. 121. Section through a single megaspore whose contour and large nucleus suggest that it is the direct product of a spore mother cell which has failed to divide.

FIGS. 122, 123. Older stages of megaspores.

FIG. 124. Median vertical section of tip of strobilus showing relation of sporangia to sporophylls and vascular system. The meristematic regions at the bases of the sporangium pedicels grow upward slightly by causing the megasporangia to appear sunken in the hollows thus formed. There were only megasporangia in this strobilus.

FIG. 125. Transverse section of strobilus through microsporangia. In the center of the figure is the axial strand surrounded by air chambers interspersed with meristematic tissue. At the right and left are two microsporangia, each showing the septum which incompletely separates it into two loculi. The rest of the diagram represents the cross sections of six sporophylls with their ligules.

FIG. 126. Apical portion of strobilus showing young sporophytes protruding from undetached megasporangia.

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